AN EVALUATION OF TECHNIQUES USED IN ESTIMATING SNAIL POPULATIONS

NELSON G. HAIRSTON, Ph.D.

Department of Zoology, University of Michigan, Ann Arbor, Mich.

BENGT HUBENDICK, D.Sc.

Curator, State Museum of Natural History, Stockholm, Sweden

JOHN M. WATSON, D.Sc., A.R.C.Sc.

Sir John Cass College, Department of Botany and Zoology, University of London

LOUIS J. OLIVIER, Ph.D.

Head, Section on Helminthic Diseases, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

SYNOPSIS

No uniform method, applicable to all situations, can be developed for the quantitative study of bilharziasis vector snail populations. The selection of survey technique and sampling device depends on the objectives of the study, the circumstances in which the work is carried out, the nature of the habitat and the resources available.

The various techniques used in obtaining snail-population estimates are divided into two categories—direct methods and indirect methods. The former involve the collection of snails from a specified habitat or for a specified period of time, while the latter include techniques, such as snail marking and palm-leaf traps, in which the snails are not obtained through the efforts of a collector. Each method and device is described in detail, and its suitability under various conditions is discussed.

Introduction

Current world-wide interest in the control of bilharziasis has focused attention upon the intermediate hosts of the causative parasite, since there is general agreement that the most promising method of controlling the disease is to eliminate or greatly reduce the numbers of these vector snails. It is necessary to obtain information about snail populations, whether the information is used for snail-control evaluation, for ecological research, or for the study of transmission potential. It is the purpose of this paper to examine the methods that have been used, or proposed, to determine the size and other characteristics of natural populations.

That it is important to obtain accurate snail-population data is not questioned by anyone in the field, but both " important " and " accurate " are relative terms in practice, and the standards of accuracy actually used have varied from one part of the world to another. One of our aims, then, is to suggest the minimum degree of accuracy that is acceptable, and another is to point out the potential errors in different methods, so that intelligent decisions can be reached as to which method to use with the time and personnel available. To a certain extent, the kind of project will determine the degree of accuracy required, but it should be pointed out that even merely reporting the presence or absence of snails implies a certain level of confidence which should not vary with changing conditions. In general, snail-population data are usually required either for judging the effect of control measures or for the pursuit of ecological studies. Although accurate data are needed for both activities, the latter is more likely to require absolute figures than the former, for which relative figures will frequently suffice. Field studies require the use of estimates, since the entire population is too large and too difficult of access to come under complete scrutiny. These estimates may be made by either direct or indirect methods, but it is emphasized that the accuracy of the method employed should be determined for the local conditions before it is put into routine application.

Technique of Survey

Before undertaking an assessment of a snail population, workers should have clearly in mind the use to which the information is to be put, and the units that are actually being assessed. Thus, under many conditions, it is desirable to discover the changes in population density, in which case the number of snails per sample is the unit to be assessed. Under other conditions, the changes in the total area infested with snails may prove more meaningful, and in that case it is the number of square or linear metres that is the unit of assessment. In perhaps the majority of cases, both units are studied. Where snail habitats are circumscribed and relatively compact, it is theoretically possible to use the entire population as the unit, but the appropriate method has never been tested on snails.

For each of the units being considered, different methods of obtaining the information have been proposed. The selection of the method or methods to be used is of great importance, because the picture of the population is distorted if the sample is not representative. For this reason, exhaustive methods should be used where possible. We recognize that such methods are time-consuming and may not be feasible for routine use. Nevertheless, they do provide one means of testing the reliability of any method that is contemplated. It is highly desirable to perform a series of tests at the beginning of a project, comparing the results from exhaustive methods with results from less time-consuming ones which are contem-

plated for routine use. Such comparisons will serve to answer the following questions:

(1) What proportion of the snails actually present is obtained by the routine method ?

(2) Is this proportion constant under the range of conditions that is likely to be encountered ?

(3) Are the different components of the population properly represented in the samples obtained by the routine method ?

(4) If the routine samples are deficient in any component of the population, is the proportion missed constant under the range of conditions that is likely to be encountered ?

If the last three questions (or the second and fourth) can be answered affirmatively, the routine method can be used with confidence, since appropriate corrections can be applied to the information obtained.

Under somewhat less ideal conditions, a method may be contemplated that cannot be tested against the kind of absolute information exhaustive collecting provides. Acceptable data may still be obtained if the method gives consistent results when tested under a variety of conditions. This approach will probably be satisfactory in situations where rather large variations in population numbers are to be expected, as, for example, where the modern molluscicides are being used in the field, or where seasonal changes are very marked.

In addition to establishing the reliability of the actual samples being taken, a programme must be planned so that the same procedure can be used over and over again without seriously altering the habitat, and can be followed in different areas between which comparisons are to be made. Relatively unskilled personnel will be performing most of the surveys, and although it is impossible to eliminate human differences in ability, the procedure should be such that it can be repeated indefinitely, without variation, by such personnel.

Snails, like most other animals in nature, are not evenly or randomly distributed even when the habitats appear uniform. With large samples, the chance of obtaining or missing a clump of snails in a single sample causes the variance to be relatively high (Olivier & Schneiderman, 1956). Therefore, it is usually better to obtain several small samples giving about the same total count of snails as a single large sample. The actual size of the sample will be determined by a number of factors. These will include the accessibility of the habitat and the snails, the density of the population, the size of the snails, and the total area from which the samples are to be taken. In general, the more accessible and the larger the snails, the larger the sample should be. The greater the density and the greater the area to be sampled, the smaller the samples should be. For most purposes, a sample size that yields an average of 3-20 snails per sample will be found

most satisfactory. With lower values, the large proportion of negative samples becomes difficult to interpret (Pesigan et al., 1958; Santos & Hairston, 1956), and with higher values it is not possible to handle properly the large numbers of snails obtained.

The pattern of sampling should be planned so that the maximum amount of information can be obtained from the results. In situations where the snail habitat is long and narrow, as along streams and irrigation canals, a linear series of samples, spaced evenly along the snail-inhabited area, is the best pattern. In wider streams, where there is a relationship between snail abundance and distance from the bank, the longitudinal transect should be replaced by a series of horizontal ones—an arrangement that can be extrapolated to include sampling over wide areas, such as rice-fields, swamps, and lakes. Ingenuity and the proper selection of tools should overcome most difficulties of accessibility, since the snails are seldom found in waters exceeding a few metres in depth. Workers should avoid the error of sampling a particularly accessible part of the habitat and assuming that they are obtaining an undistorted picture of the snail population. Whatever the sampling pattern selected, it should not be permitted to deviate on subsequent surveys, nor be influenced by the subjective judgement of the collector on the spur of the moment.

Numerical abundance is the most important information obtained from surveys, but the data can be made much more valuable by determining the age-structure of the population. Both the size and the number of whorls have been used successfully as indices of age, and any time spent on working out these relationships is well invested. From the age-structure, it is possible to estimate such important parameters as reproduction and mortality rates by the use of appropriate demographic methods (Macfadyen, 1957) and thus to anticipate changes in numerical abundance. It is important, therefore, to enumerate sizes and life-history stages separately in recording survey results.

Methods and Devices

We have classified the various methods of obtaining population estimates into two major categories: direct and indirect.

A. *Direct methods* are those in which collections are made over a specified portion of the habitat or for a specified period of time. They may be exhaustive or fractional.

1. Exhaustive techniques consist of concerted efforts to obtain all snails in a known area. They are not completely successful (Pesigan et al., 1958). All of these techniques involve removing a piece of the habitat, from which the snails are then washed free. Many of them are standard limnological methods, which, however, have never been tested in bilharziasis work. A

perusal of any standard work on limnological methods (Welch, 1948) will repay workers in the field.

(*a*) One series of devices utilizes a tube, which is pushed into the bottom mud and thus obtains a cylindrical or rectangular (Stephenson, 1947) piece of the habitat. The principal differences are in the methods employed to prevent loss of the sample in soft mud.

(i) In its simplest form, this method employs a sharpened brass pipe, 13.5 cm in diameter, which is pushed into the mud with the help of handles. The plug of habitat obtained is removed to the laboratory and washed through a series of sieves. The snails are collected from the sieves of finer mesh (16 and 20 meshes per inch, in the case of *Oncomelania quadrasi*) (Santos & Hairston, 1954, 1956). Difficulties due to soft mud can be overcome by tilting the pipe after it has been pushed into the mud and inserting a hoe or similar instrument under the open end. This device has proved most effective in the Philippines, where the vector is small, abundant, and confined to mud and shallow water (Pesigan et al., 1958). A similar technique has been used with success in Iraq, where the vector is completely aquatic (Watson, 1956). The apparatus is portable, contains no moving parts that might get out of order, and its use is well within the competence of unskilled personnel. In habitats where the water is a metre or more deep or where the snails are rather widely scattered, the technique is probably not effective, as the sample would be difficult to raise in the former case and would have to cover too large an area in the latter.

(ii) The Dendy bottom sampler (Welch, 1948) is similar to the tube sampler, but employs a different method of raising the loose substrate. The cylinder is attached by a hinge to the end of a long rod and is inverted by means of a wire or cord after being pushed into the bottom mud. A screen of appropriate mesh allows water and mud to escape, but retains any organisms that might be present. This method has not been tried in bilharziasis work, but would be more effective in deeper water than the tube sampler. Densely matted roots or aquatic vegetation might cause it to fail.

(iii) The Jenkin surface-mud sampler (Watson, 1956) consists of a heavy glass and brass tube, which is driven to the required depth into the bottom mud by weights. It then closes automatically so that an undisturbed sample of the substratum is brought to the surface. For general survey work in connexion with bilharziasis vectors, it is of little use, but where research work involves estimates of the depth at which snails have been buried, it is invaluable.

(iv) The iris diaphragm sampler (Watson, 1956). It would seem that an instrument for weed and mud sampling might well be constructed in the form of a tall strong metal cylinder, closed at the lower end by an iris

diaphragm of heavy construction operated by a rod. A cylinder of this type with an effective mouth area of 250 cm² would have an over-all diameter of about 22 cm and would thus not be unwieldy with a height of 50 cm.

(b) A second series of sampling devices utilizes hinged jaws, which bite out a known area of bottom.

(i) and (ii) The Ekman bottom sampler and the Petersen grab, which are fully described in works on freshwater biology (Welch, 1948), are too well known to warrant detailed description here. Each consists of a pair of heavy metal jaws so designed and hinged together that when the apparatus is lowered at the end of a cable it marks out a known area of the bottom, which is bitten out and enclosed in the grab when the jaws close as the grab is raised. Although of particular use in deep water, these two devices might well be more extensively employed in snail surveys than is the case at present, especially where a soft substratum is involved and investigatory work requires accuracy. Neither is suitable for use by unskilled personnel; moreover, the Petersen grab, when weighted heavily enough to cut through vegetation, is cumbersome and non-portable.

(iii) The weed-box grab is valuable in habitats where there is a dense growth of aquatic vegetation (Watson, 1956). It consists, essentially, of a large box with wire gauze sides and a steel frame in two overlapping halves, hinged together at the upper end and provided with long, strong handles. The box is thrust down into the water with the jaws wide open, thus enclosing the weeds. The handles are then pulled apart, closing the jaws of the box, which is then removed from the water containing its sample of water plants.

(c) A third type of collecting instrument which scrapes a considerable area of bottom has given encouraging preliminary results in irrigation canals in Iraq. This is the drag scoop (Hairston, 1956). It consists of a long-handled net with a deep belly, properly braced. The sample is taken by placing the net face down in the centre of the canal and dragging it across the bottom and up the side of the canal to the water's edge. Here the net is inverted and the mud is washed out. In a small series of trials, the drag scoop was found to be 5 to 200 times as effective on *Bulinus truncatus* as the dip net in standard use. It has the advantage of collecting specimens floating on the water or clinging to aquatic vegetation, as well as those resting on or buried in the bottom mud. It is most effective where banks are steep and where vegetation is not firmly rooted or is densely tangled. It would be unworkable in areas containing large stones, a criticism that applies to all the other exhaustive techniques as well.

2. Fractional techniques are those in which only part of the snails in the sampling area are obtained. Some of these methods have been used on the conscious or unconscious assumption that all the snails present were

being collected. In general they are easier to carry out than the exhaustive techniques and their principal advantage lies in the short time required to obtain the sample. Thus, more samples can always be obtained than with exhaustive techniques, and this may be an important consideration under some conditions. These techniques vary with the kind of habitat.

(a) The quadrat method is used in much ecological work. As far as snails are concerned, it is applicable only in terrestrial or semi-aquatic situations. In order to maintain a uniform sample, a metal ring is carried by the worker and dropped in the habitat. All snails inside the ring are collected. It is important that the sample be small. In the Philippines, a comparison between samples covering one square metre and samples covering 1/70 m² showed that in the latter instance approximately three times as many snails were obtained per unit area (Santos & Hairston, 1956). Because this method depends upon the worker's ability to detect snails in the habitat, it is inevitable that some are missed, including all those beneath the surface of the mud and a large proportion of small specimens less than 2.5 mm in length. Tests run by taking a tube sample in the exact spot after the quadrat collection showed that an average of 32% was added to the number of snails already obtained (Pesigan et al., 1958). The proportion was fairly consistent for different kinds of habitats, and was composed of young specimens. Quadrat sampling is impossible even in shallow water, and therefore large sections of many habitats cannot be sampled with this technique. Where it can be used, however, it is rapid and consistent, the time taken to obtain a sample being only about one-tenth of that required for one tube sampling.

(b) Dip nets and sieves are widely used in collecting aquatic snails (Abdel Azim & Ayad, 1948; Kuntz & Wells, 1951; Watson, 1951; Zakaria, 1955). The net is passed through the water and vegetation at intervals along a canal or shore-line, and sometimes the upper layer of mud is included. Results are recorded as number of snails obtained with each pass of the net. Although easy to obtain, data from such surveys are difficult to interpret, because, to our knowledge, the method has not been tested adequately against objective standards. Moreover, where water levels fluctuate by more than a few centimetres, the method gives grossly inconsistent results. In one such case, a rise in water level of less than one metre was followed by a reduction in the number of snails collected to 1/150 of the low-water figure, although abundant snails were demonstrated by another technique (Hairston, 1956). For qualitative preliminary work, the dip net is useful, and, if properly tested against one of the exhaustive methods, it might give adequate data provided water levels are stable, as they tend to be in gravity-fed irrigation systems.

(c) Counts per unit of time have been employed in a number of cases. These have one serious weakness—human frailty. The unconscious ten-

dency to collect in patches of greatest snail abundance and to select the larger individuals makes the method of doubtful value in estimating densities and of almost no value in studies involving the age-structure of the population. This is especially true in terrestrial or semi-aquatic situations, where the removal of individuals from the habitat is actually done by hand. In aquatic situations, where a tool is used, some of the objections are not valid, but the same cautions regarding constant water levels would apply here as in the case of the dip net.

(d) Fractional techniques in combination can sometimes be shown to be better than any one of them alone. Olivier & Schneiderman (1956) have used a method for estimating snail-population density in which a marked area is searched by means of a standard sieve over a measured interval of time. This, it is believed, is a more reliable method than many others that have been used. In any event, it has been critically evaluated and found to be relatively consistent in estimating snail-population density. It is urged that the method be tested in a variety of situations and compared with other methods. It is especially important that the conditions include fluctuating water levels and that the other methods compared include at least one exhaustive technique. The size of the marked-off area is of considerable importance. Better data are probably obtained if numerous small areas are searched for relatively short periods of time.

B. *Indirect methods* are those in which estimates are not based on the number of snails obtained through the efforts of the collector.

1. The recovery of marked individuals after their release in a habitat is a method that is theoretically capable of great accuracy. The population is calculated as the total number taken on a later visit, multiplied by the total number marked, and divided by the number of marked snails retaken. The difficulty with this method is that it requires ancillary information that is not easy to obtain, and depends on assumptions that are probably not valid. These assumptions are: (1) That mixing of marked snails with the general population is complete. This would require a circumscribed habitat with no immigration or emigration. (2) That the mortality rates of marked and unmarked snails are equal or negligible. Even in very stable populations of *Oncomelania quadrasi*, Santos & Hairston (1956) calculated that the mortality rates of adults varied from almost nil to 1.5% per day in different months, and that males and females differed significantly in this respect. (3) That reproduction rates are known or negligible for the period between release and recapture. This information may be extremely difficult to obtain. Santos & Hairston were unable to get absolute figures, but their index varied by a factor of 100 for different months.

It would appear obvious that if all of the information required for this method were at hand, it would probably be unnecessary to perform the

operation. We regard the technique as impracticable under most field conditions.

2. Palm-leaf traps, widely used in the Eastern Mediterranean area, are remarkably effective in determining qualitatively whether aquatic snails are present (Abdel Azim & Ayad, 1948; Stevenson, 1947). They are particularly valuable for *Bulinus*. Their most effective use would be in situations where the unit of assessment is the number of linear metres of canal infested. Quantitatively, they have little value at present. Their attractiveness to snails depends upon the stage of decay and also probably upon the abundance of other food in the vicinity. Properly standardized, palm-leaf traps might possibly yield valuable population data.

3. Laboratory colonies of snails are useful adjuncts to any ecological study. They can be used to determine features of the biology and population dynamics that are difficult or impossible to determine in the field. There are, of course, unavoidable difficulties connected with the interpretation of the information obtained. These involve the differences imposed by the artificial nature of the laboratory habitat. In general, rates of reproduction are higher and mortality is lower in the laboratory than in the field, but their analysis is useful in setting probable limits on what is likely to occur in the field.

Discussion

The rather bewildering array of techniques, devices, benefits, and cautions that we have just recounted might well discourage even the most ambitious. It has not been our intention to confuse, but to provide the thoughtful worker with a basis for deciding on the method most appropriate to his situation and needs.

Three primary considerations are involved in making the decision. These are: the nature of the habitat, the aims of the project, and the amount of resources available in personnel, equipment, and time. These three considerations will be discussed separately.

1. The habitats of the Oriental vectors *(Oncomelania)* lie on the borderline between the aquatic and the terrestrial, and are thus markedly different from the habitats of the other bilharziasis snails. These small amphibious snails occur in dense concentrations, averaging between 50 and 1000 per square metre. Small samples covering no more than $1/50$ m^2 will usually give excellent results if the sampling pattern is intelligently planned and 30 or more samples are taken in each habitat. The choice of method lies between quadrat sampling (A, 2, *a* above) and tube sampling (A, 1, *a*, i), and will depend on the considerations of aims and resources discussed below.

Aquatic habitats are too varied to be discussed as a single unit. From the standpoint of population estimates, there are two major subdivisions—irrigation systems and natural waters. Irrigation systems, because of their regularity, accessibility, and relative freedom from vegetation, lend themselves to accurate and objective sampling much more than do natural waters. With appropriate methods, the real density of snails per linear metre of canal should be obtainable. Palm-leaf traps (B, 2) are excellent for obtaining qualitative information, but quantitative methods have not been adequately tested. Most of the exhaustive techniques should be effective, particularly the drag scoop (A, 1, c), the tube sampler (A, 1, a, i), and the Dendy bottom sampler (A, 1, a, ii). The other exhaustive techniques require skill beyond that of the average field technician, but the Ekman and Petersen grabs (A, 1, b, i and ii) should be useful in canals that are too large for other methods. The dip net (A, 2, b) should be tested critically or relegated to qualitative work.

Natural waters represent the most difficult situation for sampling techniques. In shallow, accessible water, the weed-box grab (A, 1, b, iii) or the drag scoop (A, 1, c) should give good results, but the latter is suited only to situations where banks are steep. In less accessible locations, the method of Olivier & Schneiderman (1956) combining standardized collecting for a unit of time in a marked area (A, 2, d) is recommended, especially where conditions do not change drastically. For large bodies of water where boats must be used, the standard limnological tools (A, 1, b, i and ii) should be seriously considered in preference to limited sampling of the shore-line, which should be sampled separately.

2. The aims of a project may vary from a modest estimate of the effectiveness of control measures to a detailed ecological study involving population dynamics and the relative importance of different species of vectors. For the simple control programme, only three items are of real concern: the size of the snail-infested area, the effectiveness of the measure used, and the frequency with which it has to be repeated. Inspection or palm-leaf traps will solve the first problem, and the easier quantitative techniques will suffice to answer the other two questions. Where detailed ecological studies are to be undertaken, the exhaustive techniques should always be the first choice. If they cannot be used as a matter of routine, they should at least be used in evaluating the method selected for routine use.

3. With regard to resources, project planners should remember that, in sampling as elsewhere, one has to pay for what one gets, and those who carry out the project must be responsible for obtaining results commensurate with the financial outlay. It is necessary to strike a balance between the aims of the project, the number of workers available, and the size of the area that is to be covered. A few workers can sometimes cover a large area, but it cannot be emphasized too strongly that for any kind of ex-

perimental work accurate data from a small area are preferable to poor approximations from a large one. The most efficient use of personnel, then, would be to keep the standards of accuracy high and to discover empirically the area that can be covered.

Conclusion

It is obvious from the foregoing presentation that it will be impossible to develop a uniform method for the quantitative study of snail populations which will be usable in all situations. A number of different methods will be useful and the choice of method will be determined by the objectives of the study, the circumstances under which the work is to be done, the nature of the habitat, the facilities available, etc.

The points to be emphasized are: (1) that snail populations must be studied by quantitative methods, and (2) that, whatever the method chosen, the worker should evaluate his techniques and methods objectively and report the results of his evaluation. This will permit him to compare different methods in the light of his own experience and will also enable others to compare their results with his.

RÉSUMÉ

De nombreuses méthodes ont été proposées pour évaluer la densité des populations de mollusques, hôtes de schistosomes. Pour choisir la meilleure on tiendra compte de la nature de l'habitat, du résultat cherché (simple évaluation ou étude écologique approfondie), des fonds, du personnel et du temps dont on dispose.

Les auteurs décrivent les diverses techniques et les multiples appareils mis au point pour attirer, capturer et récolter les mollusques dans leurs divers habitats (tubes, tamis, filets, feuilles-appât, etc.) Les méthodes indirectes par marquage sont peu efficace sur le terrain, quoique théoriquement intéressantes. Des études des populations de mollusques en laboratoire peuvent être d'un grand secours.

S'il s'agit de populations denses (50-1000 exemplaires par m²) telles que celles d'*Oncomelania*, mi-aquatiques, mi-terrestres, un simple échantillonnage de 30 prises par habitat donnera une idée suffisante. Les habitats aquatiques ne peuvent être abordés tous de la même manière et les techniques diffèrent selon qu'il s'agit d'eau de canaux d'irrigation ou d'eaux naturelles, qui présentent le maximum de difficultés.

REFERENCES

Abdel Azim, M. & Ayad, N. (1948) *Trans. roy. Soc. trop. Med. Hyg.*, **42**, 231
Hairston, N. G. (1956) *Essay on sampling techniques* (Unpublished working document WHO/Bil. Ecol. /11)
Kuntz, R. E. & Wells, W. H. (1951) *Amer. J. trop. Med.*, **31**, 784
Macfadyen, A. (1957) *Animal ecology. Aims and methods*, London
Olivier, L. & Schneiderman, M. (1956) *Exp. Parasit.*, **5**, 109
Pesigan, T. P., Hairston, N. G., Jauregui, J.J., Garcia, E. G., Santos, A.T., Santos, B.C. & Besa, A. A. (1958) *Bull. Wld Hlth Org.*, **18**, 481

Santos, B. C. & Hairston, N. G. (1954) *Quarterly and Annual Field Reports of the Philippine Schistosomiasis Project*, Palo, Leyte (mimeographed document)

Santos, B. C. & Hairston, N. G. (1956) *Quarterly and Annual Field Reports of the Philippine Schistosomiasis Project*, Palo, Leyte (mimeographed document)

Stephenson, R. W. (1947) *Trans. roy. Soc. trop. Med. Hyg.*, **40**, 479

Watson, J. M. (1951) *J. roy. Fac. Med. Iraq*, **15**, 33

Watson, J. M. (1956) *Quantitative methods of measuring density of populations of molluscs* (Unpublished working document WHO/Bil. Ecol./21)

Welch, P. S. (1948) *Limnological methods*, Philadelphia

Zakaria, H. (1955) *Bull. endem. Dis.*, **1**, 1